



## THE POWER OF MOLECULAR DIAGNOSTICS IN THE PALM OF YOUR HAND®

### ASSAY PRINCIPLES

*brewPRO yeast* is a real-time PCR assay for the detection of wild yeast species capable of causing spoilage in brewery products. The assay utilizes a multiplex detection method which targets total *Saccharomyces cerevisiae* var. *diastaticus* on the FAM channel, *Dekkera* species on the ROX channel and *Brettanomyces bruxellensis* on the Cy5 channel. Additionally, the assay has an internal amplification control (IAC) on the HEX channel. *brewPRO yeast* couples the advantages of the real-time format with a streamlined sampling protocol eliminating the need for DNA purification and provides same day results in under 4 hours.

### INTENDED USER

*brewPRO yeast* is intended for use by personnel familiar with basic sample collection and preparation techniques associated with spoilage organism detection during production and packaging. *brewPRO yeast* is specifically designed to be easy-to-use and eliminate the need for advanced training in molecular biology.

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## MATERIALS PROVIDED

1. IS *brewPRO yeast* PCR Reagent – Cat No. IS0578
2. IS *brewPRO yeast* DIGEST Reagent – Cat No. IS0717
3. IS Buffer ACB – Cat. No. IS0714

## MATERIALS NEEDED

1. Roche LC480 II Real-Time PCR Instrument or comparable instrument capable of detecting FAM, HEX, ROX and Cy5 fluorophores
2. Centrifuge compatible with 50 mL conical tubes, capable of 1800 x g
3. Pipettes and tips capable of 5 µL and 50-500 µL volume transfers
4. 50 mL conical tubes (capable of withstanding 1800 x g centrifuge speed)

## STORAGE OF MATERIALS

The *brewPRO yeast* Buffer ACB should be stored at room temperature (20°-25°C). The *brewPRO yeast* DIGEST and PCR tubes should be stored at -20°C ± 2°C.

## PRECAUTIONS

1. Assay users should observe standard microbiological practices and safety precautions when performing this assay.
2. Do not use *brewPRO yeast* kit past indicated expiration date.
3. Deviations from the assay protocol may impact overall test performance.

## BEER SAMPLE PREP and PCR

1. Transfer 25 mL of beer sample to a 50 mL conical tube.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 1800 x g.
3. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
4. Resuspend pellet in 50 mL conical tube with 250 µL of IS Buffer ACB. Mix until the pellet is no longer visible.
5. Transfer 50 µL from the 50 mL conical tube to thawed Digest tube.
  - a. Note: Open Digest tube only when adding sample and promptly close after, to avoid cross contamination between Digest tubes.
6. Place Digest tube from Step 5 into Real-Time PCR instrument, select "BrewPRO yeast DIGEST" program and start program as directed by the Real-Time PCR User Guide (Appendix 1).
7. Upon completion of "brewPRO yeast DIGEST" program, **immediately** remove Digest tube from Real-Time PCR instrument.
8. Transfer 5 µL from Digest tube generated in Step 7 to PCR tube.

IMPORTANT:

  - a. DO NOT collect 5 µL from the bottom of the Digest tube or disturb settled contents of the Digest tube. Target DNA is present in solution at the top of the tube.
  - b. OPEN PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
9. Place PCR Tube into Real-Time PCR instrument, select "brewPRO yeast PCR" program and start program as directed by the Real-Time PCR instrument User Guide (Appendix 1).
10. Upon completion of "brewPRO yeast PCR" program, refer to Appendices 1 and 3 for results interpretation.

## COLONY SAMPLE PREP and PCR

1. Pick and transfer colony into a 1.5 mL microcentrifuge tube containing 500 µL of dH<sub>2</sub>O.
2. Mix contents by pipetting sample up and down or by vortexing.
3. Transfer 5 µL of colony re-suspension to PCR tube.
  - a. Note: Open PCR tube only when adding sample and promptly close after to avoid cross contamination between tubes.
4. Initiate amplification program as outlined in Appendix 1.

## Appendix 1: Roche LC 480II REAL-TIME PCR USER GUIDE

### DIGEST

1. Place Digest tube into the LC480 II using the LightCycler 8-Tube Strip Adapter Plate.
2. Select "New Experiment from Template" in the LC480 II Overview window and choose the "brewPRO yeast DIGEST" program.
3. Save the experiment and hit "START RUN" in the Experiment module.
4. Once the run is complete, **immediately** remove Digest tubes from the LC480 II and proceed to PCR amplification, as directed in "Beer Sample Prep and PCR" section.

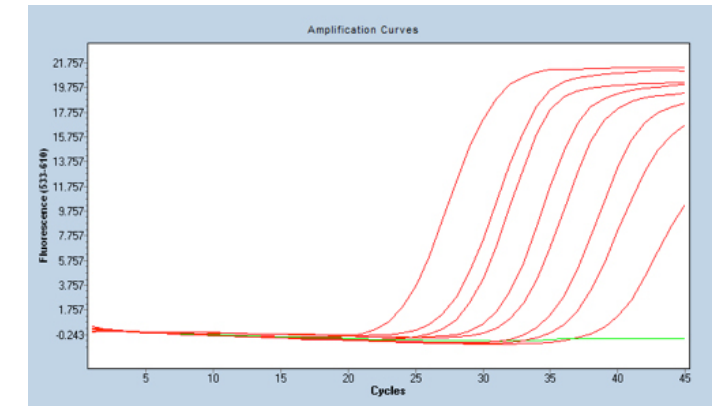
### AMPLIFICATION

1. Place *brewPRO yeast* PCR tube into the LC480 II using the LightCycler 8-Tube Strip Adapter Plate.
2. Select "New Experiment from Template" in the LC480 II Overview window and choose the brewPRO yeast program.
3. Enter sample ID's into the Sample Editor module, utilizing the Sample Subset module to indicate wells, if desired.
4. Save the experiment and hit "START RUN" in the Experiment module.
5. Once the run is complete, proceed to the Analysis module. Create a New Analysis by selecting "Abs Quant/2nd Derivative Max" from the list of analysis options provided. If samples are organized as a subset, select the appropriate sample subset from the dropdown menu.
6. Apply color compensation to eliminate signal crosstalk by selecting "In Database" from the Color Comp dropdown menu. Select the brewPRO yeast Color Compensation object from the database. Once prompted, ensure that the FAM, HEX, ROX and Cy5 channels are selected.
7. Press "Calculate" to obtain the Ct/Cp values. Inspect the traces for the characteristic exponential amplification curve shape (see Appendix 3).
8. The sample Ct/Cp results table from each channel can be exported as a text file by right clicking on the sample results table. These files can be opened in Microsoft Excel to record, organize and evaluate the data.
9. Perform Step 7-8 for each channel by toggling to each individual filter using the "Filter Comb" Button.

## APPENDIX 2: ASSAY APPLICABILITY

*brewPRO yeast* has been validated for the detection of wild yeast species capable of causing spoilage in beer. It is intended to be used on final brewery products.

## APPENDIX 3: RESULTS INTERPRETATION



Amplification curves have a characteristic shape consisting of an initial lag phase, exponential amplification phase and a final plateau phase. The final plateau phase, which represents a decrease in reaction efficiency as reagents are consumed, may not be reached in reactions containing low levels of target organisms. Amplification curves that deviate from the characteristic shape should be interpreted with caution. For each *brewPRO yeast* reaction, the cycle at which fluorescence signal rises above background fluorescence is determined and is called the "threshold cycle" (Ct) or "crossing point" (Cp), depending on the instrument. The Ct/Cp will occur at an earlier cycle for samples containing high levels of target organisms and will be delayed for reactions containing low levels of target organisms. The FAM channel is designed to detect total *S. cerevisiae var. diastaticus* spoilage organisms; the ROX channel detects total *Dekkera* species contamination, and the Cy5 channel detects *Brettanomyces bruxellensis*. The HEX channel serves as an internal amplification control (IAC) to indicate a successful PCR reaction and should be detected at a Ct/Cp value between ~26-30 cycles.

## APPENDIX 4: CONFIRMATION OF RESULTS

Presumptive positive samples can be confirmed by plating and colony PCR.

## APPENDIX 5: DISPOSAL

Invisible Sentinel PCR tubes are for single use only. Decontaminate all surfaces, media and reagents and discard in accordance with local, state, and federal regulations.

## CUSTOMER SERVICE

Invisible Sentinel customer service and technical assistance can be reached Monday-Friday between 9 AM and 5 PM Eastern Standard Time by calling 215-966-6118 and asking for an Invisible Sentinel sales or technical representative. Training on this product and all Invisible Sentinel test kits is available.

## SDS INFORMATION AVAILABLE

Safety Data Sheets (SDS) are available for this test kit and all of Invisible Sentinel's test kits by calling Invisible Sentinel at 215-966-6118.